

## Review

### **An update on diversity of sea cucumbers (Echinodermata: Holothuroidea) in Malaysia**

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Sea cucumbers (Echinodermata: Holothuroidea) are regarded as an important part of Malaysia's marine heritage due to their diversity and commercial value. Several ecological studies have been conducted aimed at documenting the distribution of sea cucumbers in Malaysia. It is estimated that more than 80 species of sea cucumber are present in Malaysia. Nevertheless, a large number of undetermined species have been recorded, thus requiring further research to update the species identification. Molecular approaches are capable of providing insights into the phylogeny of sea cucumbers and can support the outcomes of traditional morphological approaches, and a number of molecular ecological studies have been recorded in Malaysia since 1999.

**Key words.** Sea cucumbers, Malaysia, species presence, distribution, morphology, molecular ecology

## INTRODUCTION

### *Sea Cucumbers*

Class Holothuroidea is deemed a high-diversity group of the phylum Echinodermata and occurs in almost every marine environment (Kerr and Kim 2001, Kerr et al. 2005). Unique characteristics distinguishing sea cucumber (aka sea cuke) from other echinoderms include bilaterally symmetrical, worm-shaped, soft-bodied with calcareous ossicles and lacking-arms (Pechenik 2000). A few species have evolved respiratory trees. The presence of haemoglobin is another unique feature of sea cucumbers (Lambert 1997) and some species of Holothuroidea have Cuvierian tubules, unique internal organs that are extruded when disturbed in order to ward off the enemies.

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Other common names for sea cucumber are holothuroid and holothurian. In Malaysia, sea cucumber is locally known as *timun laut*, *bat*, *balat*, *brunok* and *gamat*. Among the Chinese community in Malaysia, sea cucumber is also known as *hoi sum*. Malaysia is among the top 12 mega diversity countries in the world and possesses a diversity of marine organism, including sea cucumbers. Of the species found in Malaysia, *gamat* is well known in traditional medicine industry (Ridzwan 1993) i.e. for industrial production of *gamat* oil and *gamat* water in Peninsular Malaysia, West Malaysia. *Gamat* is a local name given to species from genus *Stichopus* such as *S. herrmanni* Semper, 1868; *S. chloronotus* Brandt, 1835; *S. horrens* Selenka, 1867 and *S. ocellatus* Massin, Zulfigar, Hwai & Boss, 2002. The same common name is used by Sabah and Sarawak residents in East Malaysia. Other species of genera of *Holothuria*, *Actinopyga*, *Bohadschia*, *Pearsonothuria* and *Thelenota* for instances are locally known as *bat*, *balat*, *brunok* and *timun laut*. The names of *bat* and *balat* are commonly used by Sabah residents (Ridzwan 1993). In contrast to the economic activities in Peninsular Malaysia, Sabah is well known in food processing industry of beche-de-mer or *trepang* (i.e. dry tunics). Basically, there are two main economic activities or values of sea cucumbers in Malaysia: In Sabah they are an important resource for the food industry, while in Peninsular Malaysia they are an important resource for traditional medicine as well as modern medicine.

In general, as many as six orders of Holothuroidea namely Apodida, Elasipodida, Aspidochirotida, Molpadiida, Dendrochirotida and Dactylochirotida have been identified worldwide to date, with approximately 1400 to 2000 species distribute among the orders. Morphologically, the classification of sea cucumber to species level is normally based on the shapes of calcareous skin ossicle or spicule shape (e.g. table, cup, perforated plate, button, anchor and plate, wheel and basket), types of tentacle (e.g. dendritic, peltate, digitate and pinnate) and types of calcareous ring. Closely related species have very similar ossicles if they have recently evolved from a common ancestor (Lambert 1997). Apart from morphological characteristics or external characters, the classification of sea cucumbers into each of the six orders is also based on habitat and behaviours, as mentioned by Kerr (2000) based on the description by Pawson (1982) and Smiley (1994).

### *Overview of Taxonomy*

#### *Order Aspidochirotida*

This order consists of approximately 340 species in 35 genera and three families. Most members can be found in shallow water, and one family is restricted to the deep seawater. Shield-shaped tentacles, the presence of respiratory trees, soft and pliant body wall are among its general characteristics. It does not have posterior projections on the calcareous ring.

#### *Order Molpadiida*

This order is generally recognised by the presence of a respiratory tree, simple

tentacles and the absence of posterior projections on the calcareous ring. The body wall is soft and pliant in general. As with order Aspidochirotida, most members can be found in shallow water, and one family is restricted to the deep sea. About 95 species in 11 genera and four families make up this order. Statocyst is a special balance organ possessed by *Molpadia intermedia* (family Molpadiidae) to detect whether it is upright or not (Lambert 1997).

#### *Order Apodida*

This order comprises about 269 species in 32 genera and three families. Unlike order Aspidochirotida and order Molpadiida, respiratory tree is absent in this order. Species have digitate, pinnate and simple tentacles (in small species) with completely absent tube feet. The calcareous rings do not have a posterior projection and the body wall is often transparent and thin. As with order Aspidochirotida and order Molpadiida, most members can be found in shallow water, and one family is restricted to the deep sea.

#### *Order Elasipodida*

This order contains approximately 141 species in 24 genera and five families. Like the order Dactylochirotida, most species in this order live in deep seawater. Species have shield-shaped tentacles for shoveling sediment, a respiratory tree and the absence of posterior projection in calcareous ring. The body wall is soft to gelatinous except for family Deimatidae.

#### *Order Dactylochirotida*

There are about 35 species in seven genera and three families under this order, and most species live burrowed in soft sediment in deep seawater. One of the unique characteristics is it has muscles for retracting the oral introvert. Species are “U” shaped and have a rigid body encased in enlarged flattened ossicles, a respiratory tree, simple tentacles with a few small digits and the calcareous ring does not have a posterior projection.

#### *Order Dendrochirotida*

This order contains up to 550 species in 90 genera and seven families and thus is the most diverse. The tentacles are highly branched and extended in filtering material from the water column. The existence of members having a calcareous ring composed of numerous small pieces or having long posterior extensions distinguishes it from the other orders. Like the order Dactylochirotida, it possesses muscles for retracting the oral introvert. The body wall may be hardened from the enlargement of plate-like ossicles. Most members live either attached to hard bottoms or burrow in soft sediment in shallow seawater.

The order Aspidochirotida is currently the most diverse in terms of the number of species within the Indo-Pacific region (Allen and Steene 2002). Likewise, it has been reported that order Aspidochirotida has the highest diversity in Malaysia (Kamarul Rahim et al. 2006b, Kamarul Rahim et al. 2009). Kamarul Rahim and Ridzwan (2005) reported that five genera namely *Holothuria*, *Stichopus*, *Bohadschia*, *Thelenota* and *Actinopyga* make up the big order Aspidochirotida in Malaysian coastal areas. Lane et al. (2000) also mentioned the presence of nine species of order Dendrochirotida in coastal region of Malaysia. Out of the six orders, most members of Elapsochiroidea and Dactylochirotida live in deep sea and therefore are rarely surveyed in coastal waters.

## DISTRIBUTION OF SPECIES IN MALAYSIA

Several studies related to sea cucumbers (Echinodermata: Holothuroidea) in Malaysia have been published since 1985. The studies were conducted at several sites in Peninsular Malaysia and Sabah. Surprisingly, no reports on the species distribution of sea cucumbers in Sarawak could be found to date. Most of the studies were done using morphological characteristics as the main method for species identification.

Early studies in Malaysia on the distribution of sea cucumbers using morphological characteristics were conducted by Ridzwan and Che Bashah (1985), George and George (1987) and Ridzwan (1987), followed by Kaswandi et al. (1990), Ridzwan (1993), Ridzwan and Kaswandi (1995), Ridzwan et al. (1995), Kaswandi et al. (1995), Ridzwan et al. (1996), Ridzwan et al. (1998a) and Ridzwan et al. (1998b) and focused on Sabah. George and George (1987) found that *Colochirus robustus* Östergren, 1898 was a common species around the Bodgaya Islands in Sabah. Ridzwan (1993) subsequently identified 23 species of sea cucumber in Sabah. Out of these, eight species had local names but were undescribed scientifically, including five species of *Holothuria* and one species each of *Stichopus*, *Actinopyga* and *Molpadia* from order Molpadiida. Baine and Forbes (1998) listed 23 species of sea cucumber observed at a few locations in Peninsular Malaysia with six undetermined species. In addition, Forbes and Ilias (1999) described further 14 species with six species still requiring further verification. In Pulau Besar, Johor, Peninsular Malaysia Siti et al. (1999a) observed three genera and seven species of sea cucumber in the study area with four species from the genus *Stichopus* yet to be identified, and *Stichopus* was considered as the most abundant genus. Other related publications include Siti et al. (1999b) from Johor, and studies on the biodiversity of sea cucumber in the South China Sea by Zulfigar and Tan (1999) and Zulfigar et al. (2000b). Lane et al. (2000) subsequently documented nine species of order Dendrochirotida from the North West coast of Borneo and Peninsular Malaysia. In the marine park in Pulau Payar, located in the northwest coast of Peninsular Malaysia, Zainuddin and Forbes (2000) noted that *Holothuria* (*Halodeima*) *atra* Jaeger, 1833 was the most abundant species, and followed by *Holothuria* (*Mertensiothuria*) *leucospilota* (Brandt, 1835), *S. chloronotus* and *S.*

*horrens*. Seven species of sea cucumber were later recorded in the Balik Pulau District, Penang, Malaysia (Zaidnuddin 2002). Kamarul Rahim and Ridzwan (2005) found that southern part of Sabah, around Semporna, showed the highest diversity of sea cucumbers, and they speculated that the proximity of Sabah to the Wallace's Line might be one of the factors contributing to this interesting phenomenon. Zulfigar et al. (2007) and Sim et al. (2008) described the assemblage of sea cucumbers from Pulau Aur, Johor, Peninsular Malaysia, and the diversity and abundance of sea cucumbers from the archipelagos of Beting Patinggi Ali, Beting Raja Jarom and Pulau Layang –layang, in the South China Sea were recorded by Sim et al. (2009). Zulfigar et al. (2007) documented three families, eight genera and 20 species of sea cucumbers in 13 locations surveyed in Pulau Aur, Pulau Dayang, Pulau Lang and Pulau Pinang; by which the most abundant family was Holothuriidae (12 species) and the most abundant species were *Holothuria* (*Halodeima*) *edulis* Lesson, 1830 and *S. chloronotus*. Finally, Zulfigar et al. (2008) added some further details on the diversity of sea cucumbers in Malaysia.

Meanwhile, Tang et al. (2000), Nadirah et al. (2000a), Nadirah et al. (2000b) and Ridzwan (2000) discussed the importance of sea cucumbers as a seafood source in Sabah, and they described the survival and growth rates of selected *Stichopus* species. Ridzwan (2007) also later mentioned the importance of sea cucumbers as a potential source of health food in Malaysia and worldwide. Other studies include those by Hawa et al. (2001), Hing et al. (2002) and Hawa et al. (2004) who used electron microscopic scanning to study the calcareous rings of selected Malaysian sea cucumber species.

Several efforts have been carried out to verify and validate the taxonomic status of sea cucumbers in Malaysia. For example, Massin et al. (2002) described two new species from the genus *Stichopus* found at the Johor Marine Park, Malaysia; namely *Stichopus ocellatus* Massin, Zulfigar, Hwai & Boss, 2002 and *Stichopus rubermaculosus* Massin, Zulfigar, Hwai & Boss, 2002 which have now been accepted by the World Register of Marine Species (WoRMS - <http://www.marinespecies.org/index.php>). Zulfigar et al. (2000a) also pointed out that the specimen that was thought to be *Stichopus variegatus* for a long time in Malaysia is actually *S. horrens*, based on the colour variation and body wall patterns. However, Kamarul Rahim and Ridzwan (2005) found a large proportion of undetermined species of sea cucumber (i.e. 19 undetermined species out of total 39 species) from Sabah. The latest publication reported the presence of 50 species of sea cucumber in Malaysia from three orders and seven genera, with 34 species requiring further identification (Kamarul Rahim et al. 2009). Kamarul Rahim et al. (2009) also found that Order Aspidochirotida and genus *Holothuria* were the most diverse, and that *H. leucospilota* was the most abundant species in Malaysia.

Following revisions to the species identified by Kamarul Rahim et al. (2009), the updated species list is given in Table 1. This list includes 32 updates from that published by Kamarul Rahim et al. (2009) which are described in detail below (the species numbers refer to original list).

- 1) An addition of undetermined sp. – 1 of Order Dendrochirotida
- 2) An addition of *Stichopus rubermaculosus* Massin, Zulfigar, Hwai & Boss, 2002
- 3) *Holothuria notabilis* to *Holothuria (Lessonothuria) pardalis* Selenka, 1867
- 4) *Holothuria (Microthele) fuscogilva* to *Holothuria (Microthele) nobilis* (Selenka, 1867)
- 5) *Holothuria (Thymiosycia) impatiens* to *Holothuria* sp. – 3
- 6) *Holothuria* sp. 4 to *Polycheira rufescens* (Brandt, 1835)
- 7) *Holothuria* sp. 7 to *Holothuria (Metriatyla) ocellata* Jaeger, 1833
- 8) *Holothuria* sp. 8 to *Holothuria (Theelothuria) notabilis* Ludwig, 1875
- 9) *Holothuria* sp. 9 to undetermined sp. – 2 of Order Dendrochirotida
- 10) *Holothuria* sp. 11 to *Holothuria (Thymiosycia) aff. impatiens*
- 11) *Holothuria* sp. 12 to undetermined sp. – 2 of Order Apodida
- 12) *Holothuria* sp. 13 to *Holothuria (Metriatyla) lessoni* Massin, Uthicke, Purcell, Rowe & Samyn, 2009
- 13) *Stichopus* sp. 3 to *Thelenota anax* H.L. Clark, 1921
- 14) *Stichopus* sp. 7 to *Stichopus herrmanni* Semper, 1868
- 15) *Stichopus* sp. 6 to undetermined sp. – 3 of Order Dendrochirotida
- 16) *Synapta* sp. 1 to *Synapta maculata* (Chamisso & Eysenhardt, 1821)
- 17) *Actinopyga lecanora* to *Actinopyga lecanora* (Jaeger, 1833) – 1
- 18) *Actinopyga* sp. 2 to *Actinopyga lecanora* (Jaeger, 1833) - 8
- 19) *Actinopyga* sp. 3 to *Actinopyga lecanora* (Jaeger, 1833) - 9
- 20) *Actinopyga* sp. 5 to *Actinopyga lecanora* (Jaeger, 1833) - 10
- 21) *Actinopyga* sp. 6 to *Actinopyga lecanora* (Jaeger, 1833) - 11
- 22) *Actinopyga* sp. 7 to *Actinopyga lecanora* (Jaeger, 1833) - 12
- 23) *Actinopyga* sp. 8 to *Actinopyga lecanora* (Jaeger, 1833) - 13
- 24) *Actinopyga* sp. 9 to *Actinopyga lecanora* (Jaeger, 1833) - 2
- 25) *Actinopyga* sp. 10 to *Actinopyga lecanora* (Jaeger, 1833) - 3
- 26) *Actinopyga* sp. 11 to *Actinopyga lecanora* (Jaeger, 1833) - 4
- 27) *Actinopyga* sp. 12 to *Actinopyga lecanora* (Jaeger, 1833) - 5
- 28) *Actinopyga* sp. 13 to *Actinopyga lecanora* (Jaeger, 1833) – 6
- 29) *Actinopyga* sp. 14 to *Actinopyga lecanora* (Jaeger, 1833) - 14
- 30) *Actinopyga* sp. 15 to *Actinopyga lecanora* (Jaeger, 1833) - 15
- 31) *Actinopyga* sp. 16 to *Actinopyga lecanora* (Jaeger, 1833) – 7
- 32) *Molpadia* sp. – 1 to undetermined sp. of Order Molpadiida

The numbering of *Actinopyga lecanora* (Jaeger, 1833) i.e. from 9 to 16 is due to the same species name owned by more than one individual with different body colours. Of the 52 species listed in Table 1, 12 species are still undetermined not including all specimens identified as *Actinopyga lecanora*. The new update suggests the presence of four orders of sea cucumbers in Malaysia. It is estimated that more than 80 species of sea cucumber are present in Malaysia based on the available studies. Some of the above research reports also indirectly suggest and reveal the unclear and problematic taxonomic status of sea cucumbers in Malaysia, as there



were a large number of undetermined species recorded.

There is a lack of sea cucumber research in Sarawak. Only a statement mentioned by Ridzwan (1993) about the use of *brunok* as fishing bait was found. The written statement indirectly implies the common use of local sea cucumbers in Sarawak as an alternative for fishing activities. Lane (2005) reported that Brunei Darussalam, the adjacent Bornean country to the states of Sarawak and Sabah, had a wide diversity of sea cucumbers on the coastal reefs. They observed 14 morphospecies including *H. atra*, *H. edulis* and a few species from genus *Bohadschia*. Lane (2005) also mentioned that two out of the four *Bohadschia* species in Brunei Darussalam were suspected to be new to science. Even without documentation of the sea cucumbers in Sarawak; in the reports from Brunei Darussalam especially by Lane (2004, 2005) suggest we can expect a substantial diversity of sea cucumbers in Sarawak.

Biogeographically, Malaysia rests within the Oriental region (Huggett 1998), and the Bornean part is situated near the Wallacea Region, the zone between Wallace's Line and Weber's Line. During the Pleistocene Epoch southern Thailand, southern Indo-China, Sumatra, Java, Peninsular Malaysia and Borneo were connected by the emergent Sunda Platform (Mohsin and Ambak 1991). It is believed that the maximum lowering of sea level forming the Sunda Platform has led to the unique biogeographic distribution patterns of floras and faunas including sea cucumbers throughout South East Asia in general and Malaysia in particular (Cannon et al. 2009).

## ENVIRONMENTAL CONCERNS

In terms of environmental problems, the sudden appearance of a large number of *bat hati* from order Molpadiida along a few main beach areas in Port Dickson, Negeri Sembilan, Peninsular Malaysia, including Tanjung Gemuk beach on 5<sup>th</sup> April 2005, which was about three months after the big tsunami incident on 26<sup>th</sup> December 2004 and the earthquake at Nias Island, Sumatra, Indonesia on 29<sup>th</sup> March 2005, has slowly opened the eyes of Malaysians about the possible environmental threatens to the indigenous sea cucumbers (Tarmizi et al. 2005). However, until now there are still no conclusive experiment results explaining the real causes of such phenomena. The over-harvesting of sea cucumbers in Malaysia for economic purposes is suspected to be leading to the degradation and gradual loss of sea cucumber stock in the wild. Kamarul and Ridzwan (2005) reported a drastic decrease of sea cucumber presence and distribution in Langkawi, Kedah, Peninsular Malaysia. However, the lack of up-to-date species documentation inhibits the efforts taken by related agencies and departments such as Department of Fisheries Malaysia and Marine Park Section to protect sea cucumber resources. Hence, continuous documentation on the species presence and distribution of sea cucumbers in Malaysia is important for the monitoring, control and development of surveillance systems.

## MOLECULAR STUDIES OF SEA CUCUMBERS

Mitochondrial DNA (mtDNA) of organisms has been proven useful in addressing questions of population genetic structure, taxonomic status, conservation, zoogeography and geographic variation (Harrison 1989, Amos and Hoelzel 1992, Daniels et al. 2002). According to Endosymbiont Theory, mitochondria originated and evolved from a member of Eubacterial lineage, and the possession of 16S mitochondrial ribosomal RNA gene likely supports the theory. Overall, mtDNA consists of two ribosomal RNA regions, 13 protein-coding regions and 22 tRNAs. The genetic inheritance in mtDNA has been studied very well in understanding how species and populations respond, adapt and evolve in the natural environment (Wang et al. 2000, Wilkinson et al. 2002). Effective maternal inheritance, apparent haploid genome, non-recombination, and continuous replication are among the characteristics of mtDNA gene which make it a preferred choice for many studies in molecular ecology (Amos and Hoelzel 1992). Beside that, the rate of substitution in mtDNA is within the range of 5 to 10 times greater than in 'single-copy' nuclear DNA (Hartl and Clark 1989).

A study on the molecular phylogeny of family Cucumariidae members of sea cucumber from the Eastern Pacific by Arndt et al. (1996) based on cytochrome oxidase subunit 1 (COI) mtDNA, a protein coding region, and large ribosomal RNA subunit (18S rRNA) mtDNA, has supported the existing taxonomy depending mostly on the morphology of calcareous parts, with a few exceptions. This shows that mtDNA has the potential capability to verify and subsequently to support the taxonomic validity derived from morphological characters. mtDNA is also thought to be useful for studying conspecificity of species complex. For instance, a study on the *Bohadschia marmorata* (Jaeger, 1833) species complex in Micronesia by Clouse et al. (2005) using morphological characteristics, behaviour and mtDNA has put forward a conclusion that *B. marmorata* and *Bohadschia bivittata* (also not listed in the WoRMS database) should return to their respective status as separate species, since the morphological data as well as the phylogenetic results did not support them as sister species to each other.

12S and 16S mitochondrial rRNA gene are also among the common genes used for molecular phylogenetic studies. Such regions have been utilised to suggest strong support for an alternative taxonomic scheme to replace the existing classification, and Kerr et al. (2005) had used 16S mitochondrial rRNA gene to unravel the unclear phylogenetic relationship of sea cucumbers. According to Kerr et al. (2005), their molecular phylogeny study of sea cucumbers inferred from 16S mitochondrial rRNA gene sequences has summarised that genus *Actinopyga* and *Bohadschia* are each monophyletic and *Pearsonothuria* is a sister clade to *Bohadschia*.

Randomly Amplified Polymorphisms of DNA (RAPD) analyses on sea cucumbers carried out by Norazila et al. (1999a, 1999b, 1999c, 1999d, 2000) were the first effort in Malaysia in the use of molecular phylogenetic methods. The objectives of the studies were to find the presence of genetic variability between selected sea cucumber species from Malaysia. However, the outcomes from the



studies failed to resolve the taxonomic status of local sea cucumbers in Malaysia even at species level. The inconsistency of RAPD banding patterns might have been a factor for such weak resolution. According to Beebe and Rowe (2004), RAPD method is considered a potential and versatile molecular technique but this approach has problems of reliability and it is also susceptible to contamination. Phylogenetic analysis using DNA sequences is considered a more powerful tool, as it has the ability to study the synonymous substitution occurred between and among the nucleotide sequences.

Later molecular studies done by Kamarul Rahim et al. (2006a), Nur Hazami et al. (2006), Rosnah et al. (2006), Hajar Fauzan et al (2008), Mohd Hanafi et al. (2008), Mohd Yaman et al. (2010), Kamarul Rahim et al. (2010) and Mohd Yaman et al. (in press) supported the conclusion that mtDNA sequence analysis is a more powerful tool compared to RAPD in understanding the phylogenetic relationships of sea cucumbers in Malaysia. Kamarul Rahim et al. (2010) reported that the phylogenetic analyses of 37 partial sequences of 16S mitochondrial rRNA gene of sea cucumbers showed the presence of five main genera of sea cucumber: *Molpadia* from order Molpadiida and four genera of order Aspidochirotida namely *Holothuria*, *Stichopus*, *Bohadschia* and *Actinopyga*. They found out that the relationship of *Actinopyga* with the other genera was unclear, and that *Stichopus* was sister to *Molpadia* causing the resolution at order level to become unclear. *Stichopus* sp. 7 (Fig 1, 2 and 3) has been validated as *S. herrmanni* hence suggesting a close genetic relationship between *S. herrmanni* and *S. ocellatus*. *Stichopus variegatus herrmanni* Semper, 1868 is synonymised under *S. herrmanni* (WoRMS 2009). Beside that, there are another six updates (Fig 1, 2 and 3); namely *Holothuria notabilis* has been validated to *Holothuria (Lessonothuria) pardalis* Selenka, 1867, *Holothuria* sp. 7 to *Holothuria (Metriatyla) ocellata* Jaeger, 1833, *Holothuria* sp. 8 to *Holothuria (Theelothuria) notabilis* Ludwig, 1875, *Holothuria* sp. 11 to *Holothuria (Thymiosycia) aff. impatiens*, *Holothuria* sp. 12 to undetermined sp. – 2 of Order Apodida and *Molpadia* sp. – 1 to undetermined sp. – 1 of Order Molpadiida. Fig 1, 2 and 3 show the paraphyly of genus *Holothuria* supporting the findings of Kerr et al. (2005). This suggests that in a few cases molecular phylogeny might not be in accord with the existing taxonomy of sea cucumbers or the speculated evolutionary background. However, from another aspect, such unclear and weak taxonomic status from species level up to the highest level in Linnean classification leads to the suggestion and requirement for an alternative taxonomic scheme to replace the present classification, as suggested by Lacey et al. (2005). In other words, mtDNA is useful in revision of species validity along with the morphological approach in order to update the present taxonomy thus providing detailed information about phylogenetic relationships at several levels in the evolutionary hierarchy.

## CONCLUSIONS

Studies on species distribution of sea cucumbers in Malaysia show that there are about 76 species of sea cucumbers. It is further estimated that more than 80 species of sea cucumbers are present throughout Malaysia. In the future, further studies on

morphology and genetics of indigenous sea cucumbers from Malaysia especially for the undetermined species are required in order to get a better view and more satisfactory resolution on the taxonomy. Species identification based on the characteristics of calcareous ring, tentacle and ossicle are also important for proper species cataloging and specimen collection. In terms of sampling, more study sites need to be considered and included in the future research, especially in Sarawak to get better insights on the species distribution, and to study the possible impacts of Pleistocene event on the genetics of sea cucumbers in Malaysia. Furthermore, more molecular work such as microsatellite and phylogenetic analyses incorporating protein-coding regions of mitochondria such as cytochrome b and cytochrome c oxidase I (COI) as additional molecular approaches are important to verify the present inferences. The main problem to be tackled in the future based on the recent findings is the paraphyly of genus *Holothuria*.

In order to help maintain the stock of sea cucumbers for the trade in Langkawi, Kedah, Peninsular Malaysia, Baine and Sze (1999) have suggested that the demand in Langkawi may be supplied from three directions: from within through restocking initiatives, from Thailand in a trade agreement and from Pangkor Island, Perak, Peninsular Malaysia as part of a managed fishery. Sea cucumbers are not included in the list of endangered species in Malaysia, neither it is considered endangered by International Union for Conservation of Nature (i.e. The IUCN Red List of Threatened Species™ - <http://www.iucnredlist.org/>) which maintains a global list of endangered species. However, for the conservation of sea cucumbers in Malaysia, Mohd and Zahaitun (2004) have emphasised the need to support the inclusion of species of sea cucumber from the families of Holothuridae and Stichopodidae into the Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) as protected species. They suggest that a few considerations must be addressed and put into account before such inclusion is made especially issues pertaining to legislation and administration, the status of sea cucumber as a protected species, research into the status and level of exploitation of sea cucumber resources.

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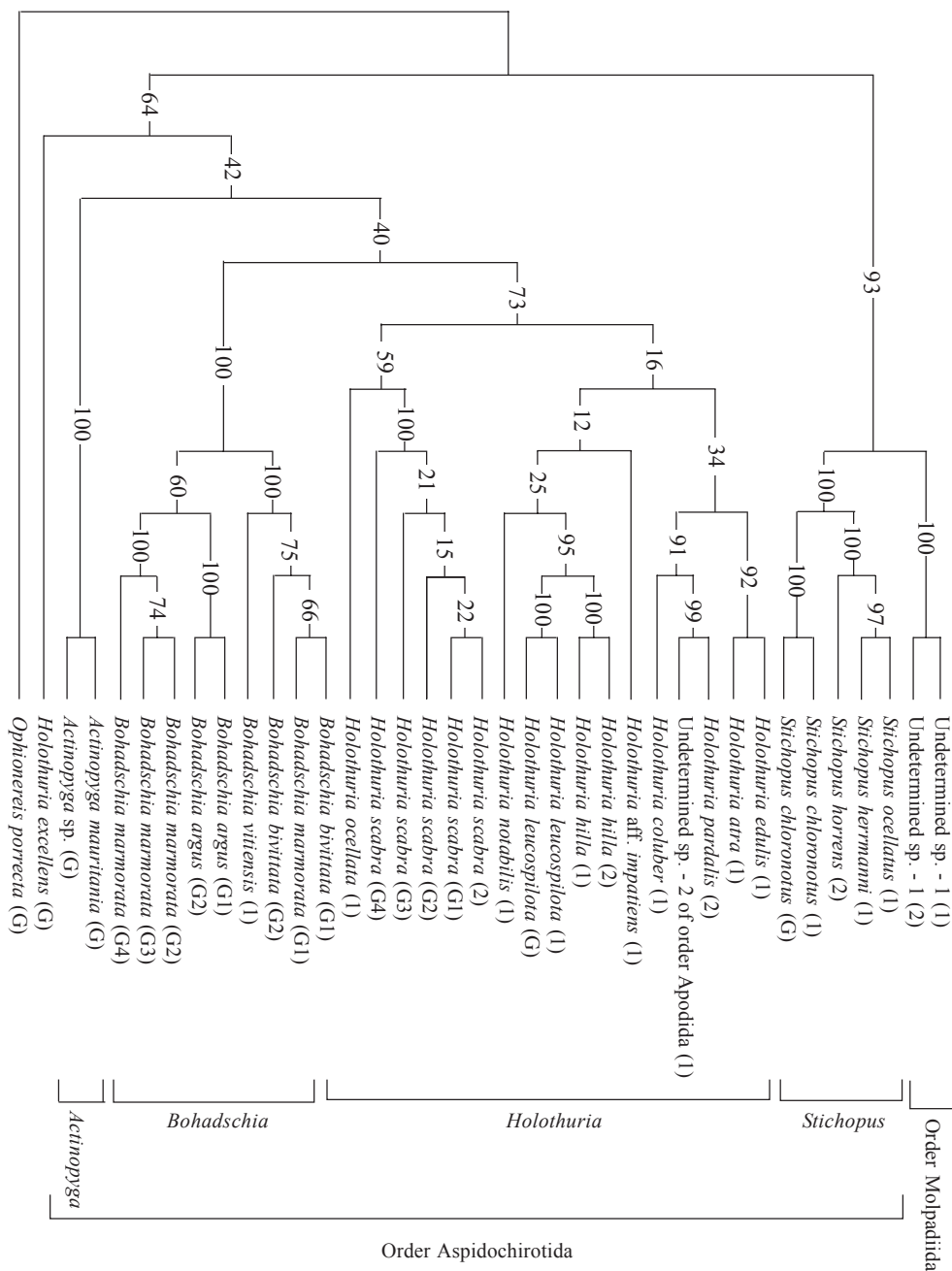


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**Table 1.** List of sea cucumbers from Malaysia. Updated from Kamarul Rahim et al. (2009). PM=Peninsular Malaysia, S=Sabah, Status (A=abundant, C=common, R=rare).

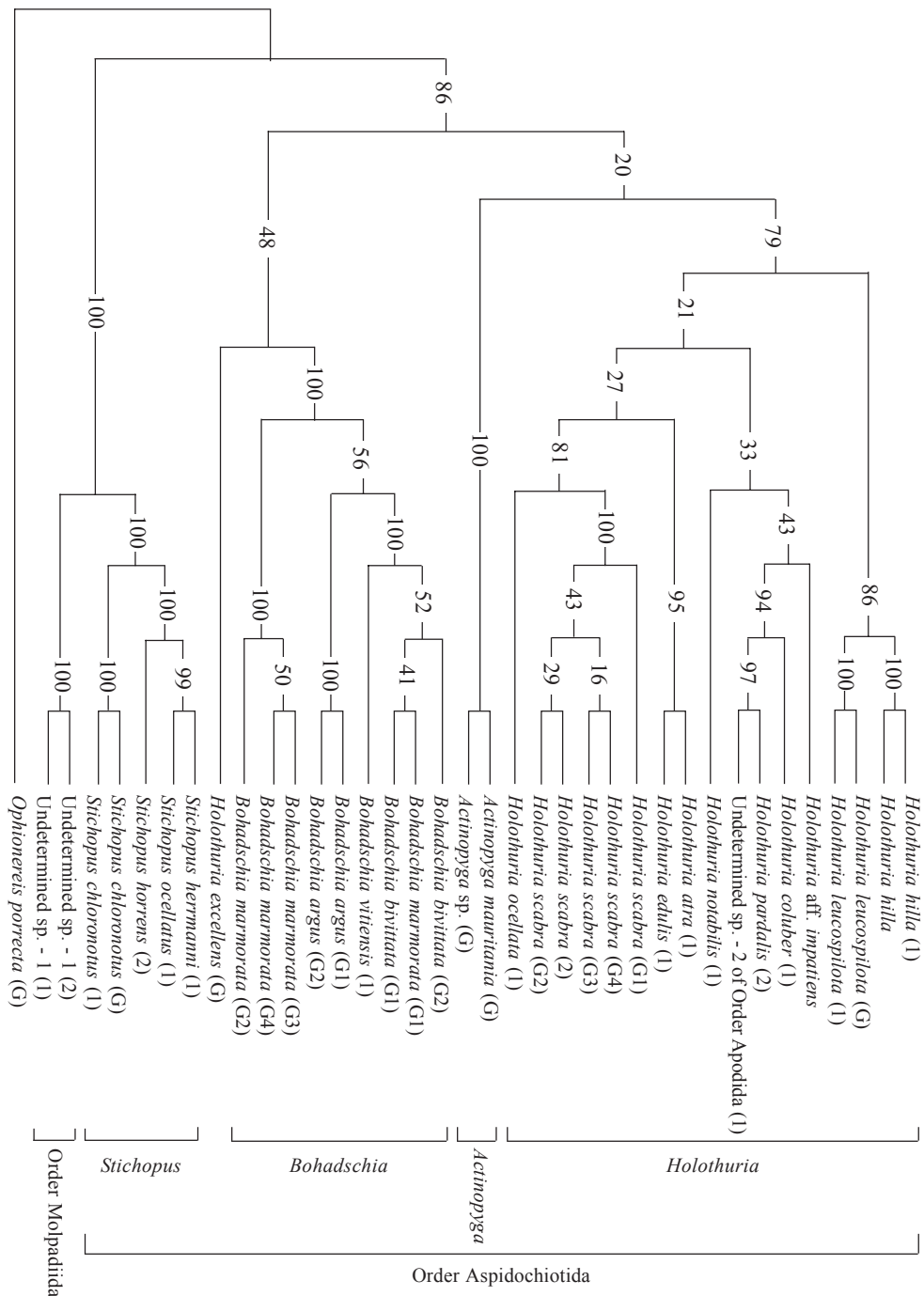
<i>Species</i>	<i>Local Name</i>	<i>PM</i>	<i>S</i>	<i>Status</i>
Order Aspidochirotida				
Family Holothuriidae				
<i>Holothuria (Mertensiothuria) leucospilota</i> (Brandt, 1835)	<i>Bat puntil / White threads fish</i>	x	x	A
<i>Holothuria (Mertensiothuria) hilla</i> Lesson, 1830	<i>Bat / Tiger tail sea cucumber</i>	x		C
<i>Holothuria (Metriatyla) scabra</i> Jaeger, 1833	<i>Bat putih / Sandfish</i>		x	R
<i>Holothuria (Metriatyla) ocellata</i> Jaeger, 1833	<i>Bat</i>	x		C
<i>Holothuria (Metriatyla) lessoni</i> Massin, Uthicke, Purcell, Rowe & Samyn, 2009	<i>Bat putan / Golden sandfish</i>		x	C
<i>Holothuria (Halodeima) atra</i> Jaeger, 1833	<i>Bat hitam / Lollyfish</i>	x	x	C
<i>Holothuria (Halodeima) edulis</i> Lesson, 1830	<i>Bat senjata anjing / Pinkfish</i>	x	x	C
<i>Holothuria (Microthele) nobilis</i> (Selenka, 1867)	<i>Bat susu / White teatfish</i>		x	R
<i>Holothuria (Microthele) fuscopunctata</i> Jaeger, 1833	<i>Bat / Elephant trunkfish</i>		x	R
<i>Holothuria (Acanthotrapeza) coluber</i> Semper, 1868	<i>Bat sumping / Snakefish</i>	x	x	C
<i>Holothuria (Lessonothuria) pardalis</i> Selenka, 1867	<i>Bat</i>	x		C
<i>Holothuria (Theelothuria) notabilis</i> Ludwig, 1875	<i>Bat</i>	x		C
<i>Holothuria (Thymiosycia) aff. impatiens</i>	<i>Bat</i>	x		C
<i>Holothuria</i> sp. - 3	<i>Bat brown</i>		x	C
<i>Holothuria</i> sp. - 6	<i>Bat kasut</i>		x	C
<i>Bohadschia argus</i> (Jaeger, 1833)	<i>Bat / Leopardfish / Tigerfish</i>		x	R
<i>Bohadschia vitiensis</i> (Semper, 1868)	<i>Bat nangka / Brown sandfish</i>	x	x	R
<i>Bohadschia</i> sp. - 1	<i>Bat</i>	x		R
<i>Bohadschia</i> sp. - 2	<i>Bat</i>	x		R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 1	<i>Bat puyuh / Stonefish</i>		x	C
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 2	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 3	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 4	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 5	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 6	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 7	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 8	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 9	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 10	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 11	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 12	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 13	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 14	<i>Bat puyuh / Stonefish</i>		x	R
<i>Actinopyga lecanora</i> (Jaeger, 1833) - 15	<i>Bat puyuh / Stonefish</i>	x		R

<i>Species</i>	<i>Local Name</i>	<i>PM</i>	<i>S</i>	<i>Status</i>
<i>Pearsonothuria graeffei</i> (Semper, 1868)	<i>Bat / Flowerfish / Blackspotted sea cucumber</i>		x	C
Family Stichopodidae				
<i>Stichopus rubermaculosus</i> Massin, Zulfigar, Hwai & Boss, 2002	<i>Gamat</i>		x	R
<i>Stichopus chloronotus</i> Brandt, 1835	<i>Talifan varieti hitam / Greenfish</i>	x	x	C
<i>Stichopus horrens</i> Selenka, 1867	<i>Gamat / Dragonfish</i>	x	x	C
<i>Stichopus ocellatus</i> Massin, Zulfigar, Hwai & Boss, 2002	<i>Gamat</i>	x	x	R
<i>Stichopus herrmanni</i> Semper, 1868	<i>Gamat / Curryfish</i>	x		R
<i>Stichopus vastus</i> Sluiter, 1887	<i>Gamat</i>	x	x	C
<i>Stichopus</i> sp. - 1	<i>Kumbatas</i>		x	R
<i>Stichopus</i> sp. - 2	<i>Kambatan</i>		x	C
<i>Thelenota anax</i> H.L. Clark, 1921	<i>Bat / Amberfish</i>		x	R
Order Molpadiida				
Undetermined sp. - 1	<i>Bat hati / Brunok</i>	x	x	C
Order Apodida				
<i>Synapta maculata</i> (Chamisso & Eysenhardt, 1821)	<i>Taliaga</i>		x	C
<i>Synapta</i> sp. - 1	<i>Taliaga</i>		x	C
<i>Polycheira rufescens</i> (Brandt, 1835)	<i>Bat</i>	x		C
Undetermined sp. - 2	<i>Bat</i>	x		R
Order Dendrochirotida				
Undetermined sp. - 1	<i>Bat</i>	x		R
Undetermined sp. - 2	<i>Bat</i>	x		R
Undetermined sp. - 3	<i>Bat</i>	x		R



**Figure 1.** Topology of neighbour joining tree (majority rule consensus tree) of sea cucumber species inferred from 16S mitochondrial ribosomal RNA gene using PAUP\* version 4.0b10 (Swofford 1998). Kimura 2-parameter evolutionary model with 1000 replications was incorporated. Abbreviation of G refers to corresponding sequences obtained from GenBank. Each partial sequence detail is described in TABLE 1. The tree was rooted with a sequence of *Ophionereis porrecta*, a brittle star (GenBank accession number: AY365184). Numbers at nodes indicate the bootstrap values in percentage (%). Modified from Kamarul Rahim et al. (2010) with seven updates to the species identification.





**Figure 3.** Topology of maximum likelihood tree (majority rule consensus tree) with molecular clock of sea cucumber species inferred from 16S mitochondrial ribosomal RNA gene using PHYLIP version 3.6b (Felsenstein 2004). Kishino-Hasegawa model was incorporated along with global rearrangements and empirical base frequencies. Abbreviation of G refers to the corresponding sequences obtained from GenBank. Each partial sequence detail is described in TABLE 1. The tree was rooted with a sequence of *Ophionereis porrecta*, a brittle star (GenBank accession number: AY365184). 1000 sequence replications and 100 data sets were used. Numbers at nodes indicate the bootstrap values in percentage (%). Modified from Kamarul Rahim et al. (2010) with seven updates to the species identification.